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Short communication

Protective effect of hyperimmune egg yolk IgY antibodies against *Eimeria tenella* and *Eimeria maxima* infections☆

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ABSTRACT

Avian coccidiosis is caused by several distinct species of *Eimeria* protozoa and is the major parasitic disease of poultry of economic importance. As an alternative strategy to control avian coccidiosis without using prophylactic medications, we have investigated the efficacy of inducing passive immunity against coccidiosis by orally feeding hyperimmune IgY antibodies. In this study, a commercially available egg yolk powder, Supracox® (SC), a purified IgY fraction of egg yolk prepared from hens hyperimmunized with three major species of *Eimeria* oocysts, were continuously fed to young chicks from hatch. Upon orally infecting these broiler chicks with *Eimeria tenella* and *Eimeria maxima* oocysts at 1 week of age, they showed significantly higher body weight gains ($P < 0.05$) compared to the untreated controls. Furthermore, SC-fed chicks showed significantly less intestinal lesions and reduced fecal oocyst output compared to the untreated controls following oral infections with *E. tenella* and *E. maxima*. These results provide clear evidence that passive immunization of chicks with hyperimmune egg yolk IgY antibodies provide significant protection against *E. tenella* or *E. maxima* infections.

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1. Introduction

Avian coccidiosis is an intestinal disease caused by several distinct species of *Eimeria* protozoa and is the most economically significant parasitic infection of the poultry industry worldwide (Lillehoj and Lillehoj, 2000). Due to increasing concerns with prophylactic drug use and high costs of vaccines, alternative control methods need to be developed. Therefore, much recent interest has been devoted toward the development of drug-independent control strategies against coccidiosis (Lillehoj and Lee, 2007a,b; Lee et al., 2007a,b,c).

An alternative control strategy potentially applicable to intestinal diseases such as avian coccidiosis may involve a passive immunization strategy using hyperimmune, pathogen-specific secretory IgY antibodies (Lee et al., 2009). For example, egg yolk IgY antibodies offer a practicable alternative to mammalian serum antibodies because of their feasibility for large-scale commercial production and the relative non-invasive methods used for their preparation (Schade et al., 2005; Yokoyama et al., 1992, 1998). Although passive transfer of maternal antibodies from hens infected with *Eimeria maxima* to eggs has been shown to partially protect offsprings against

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Eimeria tenella infection (Smith et al., 1994), and an intravenously introduced mouse monoclonal antibody against a major oocyst wall protein of *E. tenella* (Karim et al., 1996) could reduce fecal oocyst output following *E. tenella* or *E. maxima* infection, these strategies do not provide a prolonged high titer antibodies to young chicks, especially at the site of infection. Therefore, we need a delivery strategy that can sustain high-titer antibodies in the gut secretion in order to obtain an effective control method to reduce the intestinal damage due to coccidiosis. In a recent report, we have demonstrated that a continuous oral feeding of young chicks with hyperimmune IgY antibodies conferred significant protection against *Eimeria acervulina* infection (Lee et al., 2009). In this study, we hypothesized that passive immunization with egg yolk containing hyperimmune anti-coccidia IgY antibodies to young chicks would confer protection against other species of *Eimeria*. To verify this hypothesis, we continuously fed young chicks a commercially available egg yolk powder that contains high levels of hyperimmune IgY antibodies (Supracox®, SC) from hatch and the efficacy of passive immunity was evaluated by infecting these chicks orally with *E. tenella* and *E. maxima* oocysts.

2. Materials and methods

2.1. Preparation of egg yolk IgY antibodies

Egg powder containing hyper-immune egg yolk IgY (Supracox®, SC) and a control IgY were produced by IASA (Investigación Aplicada, S. A. de C. V., Puebla, Mexico) as described (Lee et al., 2009). Control egg powder was obtained from un-immunized hens. SC was prepared from egg yolks of specific-pathogen-free broiler chicks hyper-immunized with live oocysts of three major *Eimeria* species, *E. tenella*, *E. acervulina*, and *E. maxima*. Hyper-immunization of chicks were carried out by an oral infection with 4000 sporulated oocysts of *E. tenella*, *E. acervulina*, and *E. maxima* starting at 60 days of age and continuing through the egg production period. The IgY antibodies were obtained by removing the lipid and fatty components with solvents, followed by protein precipitation and purification (Avid AL, Unisyn Technologies, Tustin, CA), and spray-dry (Yokoyama et al., 1993). IgY antibodies were identified using horseradish peroxidase conjugated goat anti-IgY antibody in ELISA (Pierce Biotechnology, Rockford, IL). For each feeding study, two groups (uninfected and infected controls) were fed *ad libitum* with a standard diet alone or a standard diet supplemented with 0.01% (SC 0.01), 0.02% (SC 0.02), or 0.05% SC (SC 0.05) (v/v). These concentrations were based on the dietary ratios used in previous studies which showed a significant protective effect against *E. acervulina* infection (Lee et al., 2007c, 2009). At 7 days post-hatch, all groups except the uninfected control chicks were orally infected with 1.0×10^4 sporulated *E. tenella* or *E. maxima* oocysts.

2.2. Evaluation of protective immunity

Three independent *in vivo* trials were carried out to evaluate the efficacy of SC in protection against avian coccidiosis. Three different disease parameters of coccidiosis including body weight gain, fecal oocyst shedding, and gut lesion score were assessed as described (Johnson and Reid, 1970; Gabriel et al., 2006; Lee et al., 2008). All experiments were performed following approval by the Beltsville Agriculture Research Center Small Animal Care and Use Committee.

2.2.1. Trial 1 – body weight gain determination

One hundred, 1-day-old broilers chicks (ROSS/ROSS, Longenecker's Hatchery, Elizabethtown, PA) were used in this experiment. Fifty chicks were randomly assigned to 5 groups (10 chicks/group) in electrically heated battery units for *E. tenella* infection and the other 50 chicks were assigned to 5 groups (10 chicks/group) for *E. maxima* infection. Body weights were measured at 0 (pre-infection) and 10 days post-infection (dpi).

2.2.2. Trial 2 – determination of fecal oocyst output

For fecal oocyst enumeration, chicks from each groups (10 chicks/group) were placed in collection cages (2 chicks per collection cages) and fecal droppings were collected between 5 and 10 dpi. Pooled fecal material was suspended in 3 l of water and oocyst numbers were determined in two 35 ml samples using a McMaster chamber according to the formula (Lee et al., 2007a): total oocysts/bird = oocyst count × dilution factor × (fecal sample volume/counting chamber volume)/2.

2.2.3. Trial 3 – determination of gut lesion score

Six chicks from each group were randomly chosen for gut lesion scoring at 10 dpi. Lesion scores were based on the scoring system as described by Johnson and Reid (1970), and each chick received a numerical value from 0 to 4. Lesion scores were evaluated by 2 independent observers.

2.3. Statistical analysis

Data analyses were performed using SPSS software (SPSS 15.0 K for Windows, Chicago, IL). All data were expressed as mean ± SEM values. The ANOVA test was used to test for differences between the groups. Duncan's multiple range test or *t*-test were used to analyze differences between the mean values and differences were considered statistically significant at $P < 0.05$.

3. Results

Three experimental trials were used to assess the effectiveness of hyperimmune egg yolk IgY as a dietary supplement in conferring passive immunity against *E. tenella* and *E. maxima* infections.

The SC 0.02 and SC 0.05 groups infected with *E. tenella* exhibited increased body weight gains compared to the infected control group on the standard diet (Fig. 1A), whereas only the SC 0.05 group displayed increased weight gain following infection with *E. maxima* (Fig. 1B). In the case of *E. tenella* infection, the weight gains in the SC 0.02 and SC 0.05 groups were identical to that of the uninfected controls, whereas the weight gains of the SC 0.05 group

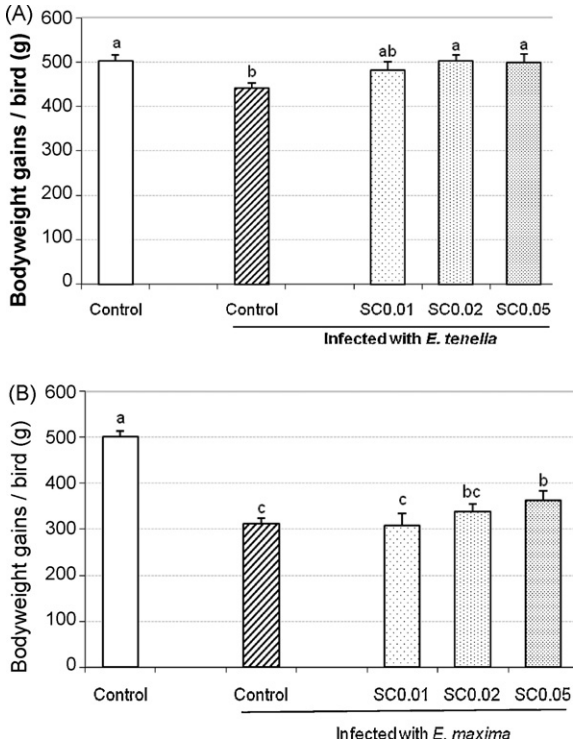

Fig. 1. Effect of oral hyperimmune IgY antibodies on body weight gain of birds infected with *E. tenella* and *E. maxima*. One-day-old broiler chickens were fed a standard diet supplemented with SC at 0% (Control), 0.01% (SC 0.01), 0.02% (SC 0.02), or 0.05% (SC 0.05) (v/v). The chickens were uninfected (Control) or were orally infected with 1.0×10^4 sporulated oocysts of *E. tenella* (A) or *E. maxima* (B) at 7 days post-hatch, and body weight gains between 0 and 10 dpi were measured. Each bar represents the mean ± SEM values ($N = 10$). Within each graph, bars not sharing the indicated letters are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

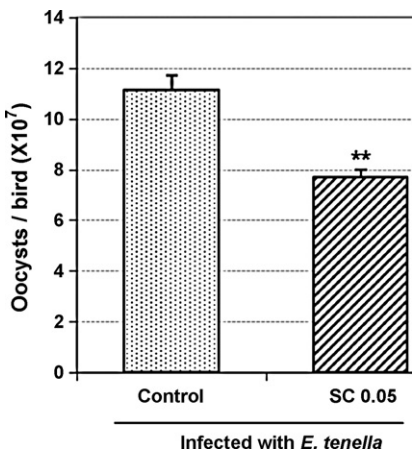

Fig. 2. Effect of oral hyperimmune IgY antibodies on oocysts shedding in birds infected with *E. tenella*. One-day-old broiler chickens were fed a standard diet supplemented with SC at 0% (Control) or 0.05% (SC 0.05) (v/v), challenged with 1.0×10^4 sporulated oocysts of *E. tenella* at 7 days post-hatch and fecal oocyst shedding between 5 and 10 dpi were measured. Each bar represents the mean ± SEM values ($N = 10$). The difference in oocyst numbers between the infected control and the SC 0.05 groups was tested by the Student's *t*-test. $^{**}P < 0.01$.

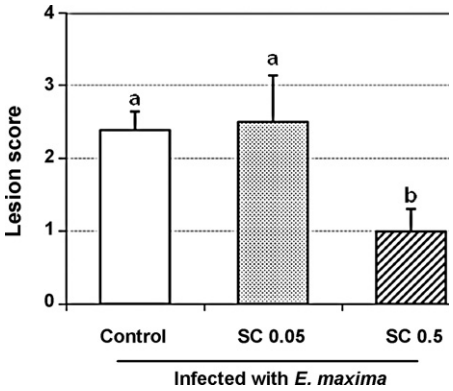

Fig. 3. Effect of oral hyperimmune IgY antibodies on lesion scores of birds infected with *E. maxima*. One-day-old broiler chickens were fed a standard diet supplemented with 0% (Control), 0.05% (SC 0.05), or 0.5% (SC 0.5) (v/v), challenged with 1.0×10^4 sporulated oocysts of *E. maxima* at 7 days post-hatch, and lesion scores were measured at 10 dpi. Each bar represents the mean ± SEM values ($N = 6$) of two independent observations. Within each graph, bars not sharing the indicated letters are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

infected with *E. maxima* remained less than that of the uninfected controls. SC 0.05 group showed significantly reduced fecal oocysts shedding following *E. tenella* infection compared to the infected chicks on the standard diet (Fig. 2). Finally, the SC 0.5 group infected with *E. maxima* exhibited decreased lesion scores compared to the infected group on the standard diet (Fig. 3).

4. Discussion

In this study, the protective effects of orally administered *Eimeria*-specific hyperimmune IgY antibodies obtained from hens hyperimmunized with mixed species of coccidia oocysts were evaluated against *E. tenella* and *E. maxima* infections. In the chicks infected with 1.0×10^4 *E. tenella* oocysts, the SC 0.05 group showed significantly increased body weight gains and reduced fecal oocyst shedding compared to the infected chickens on the standard diet. Similarly, in chickens infected with *E. maxima*, the SC 0.05 group showed significantly increased body weight gain whereas gut lesion scores were reduced only in the SC 0.5 group. We conclude that passive immunization with hyperimmune IgY antibodies provides substantial protection against avian coccidiosis in newly hatched chicks.

From a practical perspective, the infection doses of *E. tenella* and *E. maxima* that were used in this study are likely to be considerably higher than the levels that commercial broilers are exposed to in the poultry production facilities (Wallach et al., 1995). In separate field trials involving large numbers of broiler chicks carried out in two different poultry farms, continous feeding of hyperimmune IgY to broiler chicks from hatch until the grow-out period resulted in significant body weight gains compared to the conventionally raised control broiler chicks on the standard diet (data not shown). Therefore, future investigations are needed to investigate the underlying immune mechanisms mediated by hyperimmune IgY antibodies in

protection against coccidiosis. Finally, elucidating the parasite components that are recognized by antigen-specific IgY antibodies will facilitate the discovery of novel coccidiosis vaccines.

Passive induction of protective immunity mediated by oral feeding of hyperimmune egg yolk antibodies is different from other types of antibody-mediated protection which have been previously described (Belli et al., 2004). CoxAbic® (Abic Biological Laboratories Teva Ltd., Beit Shemesh, Israel) is an avian coccidiosis recombinant vaccine derived from *E. maxima* gametocytes which is used to immunize broiler hens with the intent of transferring *Eimeria*-specific immunity to offspring through the egg yolk. However, maternally derived antibodies transferred *in ovo* wanes with time and disappears within 3 weeks after hatching. In contrast, the continuous feeding of hyperimmune egg yolk IgY antibodies from hatch until the market-age will allow the maintenance of high levels of secretory IgY antibodies at the local infection site, and the level of protective antibodies can be easily sustained as long as chicks are fed the SC-supplemented diet. In addition, SC contains polyclonal antibodies which are directed against many different life cycle stages of multiple *Eimeria* species.

In conclusion, this study demonstrates that feeding of hyperimmune IgY antibodies provides significant protection against avian coccidiosis in newly hatched chicks and this passive immunization strategy may prove beneficial in the disruption of the infectious cycle of multiple *Eimeria* species. Further studies are planned to characterize the dose–response relationship between IgY in the diet and coccidiosis in the field and to elucidate its mechanism of action.

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